

## Anticonvulsant Activity of Hydroalcoholic Extract of *Ageratum Conyzoides* L. (Asteraceae) in Mice

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### Abstract

This work aims to study the anticonvulsant activity in vivo of the hydro alcoholic extract of *Ageratum conyzoides* L. (ASTERACEAE) in mice. Seizure was induced by electric stimulation of animal ears and intraperitoneal injection of picrotoxin. The extract increases the seizure threshold and reduces picrotoxin-induced convulsion duration. Animals of control group reacted to electric shock of  $10.67 \pm 0.74$  mV, versus  $14.33 \pm 0.64$ ,  $22.67 \pm 1.28$  and  $29.33 \pm 0.64$  mV for the animals treated with the extract at doses 200, 400 and 800 mg/kg respectively ( $P < 0.05$ ). Duration of clonic seizure induced by picrotoxin decreases from  $36.04 \pm 3.46$  s in control group to  $31.33 \pm 0.88$ ,  $14.08 \pm 1.02$  and  $10.07 \pm 1.31$  s in the treated animals, while tonic seizure duration in control group is equal to  $156.33 \pm 24.53$  s versus  $73.02 \pm 5.32$  s and  $29.67 \pm 3.59$  s in the animals treated with the extract at doses 400 and 800 mg/kg respectively ( $P < 0.05$ ). Alkaloids in the extract might be responsible for this activity by blocking  $\text{Na}^+$  or  $\text{K}^+$  channels, it also might enhance GABA or inhibit glutamate action.

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**Keywords:** Ageratum Conyzoides, Anticonvulsant, Electric Stimulation, Picrotoxin

## Introduction

Convulsion is a sudden and involuntary contraction of skeletal muscle due to nervous hyper sensibility and hyper synchronization of brain neurons group (Vaughan & Delanty, 2002). It often occurs during childhood, because of high temperature occurring during events such as viral infections. It can also happen as epileptic crises in children as well as in adult (Jones & Jacobsen, 2007).

Two types of neuromodulators are found in the central nervous system: stimulating ones such as glutamate and inhibiting ones such as GABA ( $\gamma$ -aminobutyric acid). This last neurotransmitter increases postsynaptic chloride influx. A disequilibrium between these two neurotransmitters in favor of glutamate or the deficiency of GABA enhances convulsion (Bertrand, 2006; Ben-Ari, 2007; Eusebio & Micallef-Roll, 2010). It is a complicated mechanism, that is why most of anticonvulsant medicines are membrane stabilizer (Wilcock & Twycross, 2011), glutamate antagonist, or influence GABA system (Deshmukh *et al.*, 2011).

*Ageratum conyzoides* L. (Asteraceae), is an annual herbaceous plant with a long history of traditional medicinal uses in several countries. It is widely used in traditional medicine by various cultures worldwide, although applications vary from region to region. In Central Africa it is used to treat pneumonia, but the most common use is to cure wounds and burns (Durodola, 1977). Traditional communities in India use this species as a bactericide, antidiarrhoea, and antihelminthic (Borthakur & Baruah, 1987), it also has insecticidal and nematocidal activity (Ming, 1999). In Cameroon and Congo, *A. conyzoides* is used to treat fever, rheumatism, headache, and colic (Menut *et al.*, 1993; Bioka *et al.*, 1993). An ethnopharmacological survey that we have conducted among the population of Andasibe (Madagascar) revealed its use to prevent seizure crisis. Decoction of the whole plant is regularly given to children who have convulsion problem.

The objective of this study is to evaluate the anticonvulsant activity of the hydroalcoholic extract of *A. conyzoides*. Different methods, for example, transcranial neurostimulation (Sayyah *et al.*, 2011), or injection of convulsion-inducing drugs such as pentilene tetrazole, picrotoxin (Löscher & Schmidt, 1988; Visweswari G. *et al.*, 2010), pilocarpine (Cavalheiro *et al.*, 1982), or bicuculline (Johnston, 2013), can be used to evaluate the anticonvulsant activity of a product. In this study electric shock and i.p., injection of picrotoxin were used to induce convulsion in mice.

## **Materials And Methods**

### **1. Extraction and phytochemical screening**

The entire *A. conyzoides* plant was collected at Andasibe (Madagascar) and a voucher specimen was identified at the herbarium of Parc Botanique et Zoologique de Tsimbazaza (PBZT) (Antananarivo, Madagascar). The plant was dried in shade, at room temperature, then ground with a grinder “BROOK CROMPTON ©, Série 2000”. The powder was macerated in a mixture of ethanol and water (60:40), at room temperature, for 3 days. The macerate was filtrated and evaporated to dryness with a vacuum evaporator, Rotavapor (BÜCHI), at 80°C. The dry extract was dissolved in distilled water for the anticonvulsant tests. Phytochemical screening was carried out to determine the main chemicals in the extract. Specific reagents were used, they react with specific chemical family to give a characteristic color or precipitation. Dragendorff and Wagner reagents were used to detect alkaloids, Fehling liquor for reducing sugars, chlorhydric acid for anthocyanins, gelatin for tannins, sulfuric acid and magnesium ribbon for flavonoids, and Salkowski reaction using concentrated sulfuric acid and Lieberman Burchard, using anhydrous acetic and sulfuric acid for steroids and terpenoids (Fong *et al.*, 1977). All reagents used in this study were prepared using chemicals from Sigma-Aldrich ®.

### **2. Experiment animals**

Male mice of Swiss strain aged 6 to 8 weeks and weighing between 20 and 25 g were used in this experiment. The animals were fastened for 12 h prior to the test and divided into 4 groups of 6 animals per group, 1 control group which received 10 ml/kg of distilled water, and the 3 other groups were treated with the extract at doses 200, 400 and 800 mg/kg in 10 ml/kg distilled water, respectively, administered orally.

The experiments were conducted following the guidelines of the ethic committee of Sciences Faculty, University of Antananarivo, Madagascar (Ref: CE/Fac Sciences/Pharmacol./001, 02/21/2019).

### **3. Study of *A. conyzoides* activity on convulsion threshold**

One hour after oral administration of the extract and water, electrodes rubbed with conductive gel (TransCard ®) were fixed on the ear lobe of the animals. A neurostimulator (Harvard®) was used to deliver an electric shock of 20 ms width and 60Hz square wave to the mice's ear lobe. To determine the seizure threshold, the stimulation was started at 5 mV and increased until the animal reacted to the stimulation (Branco *et al.*, 2009; Gerstner *et al.*, 2014).

### **4. Study of *A. conyzoides* activity on picrotoxin-induced convulsion**

*A. conyzoides* hydroalcoholic extract activity was studied on picrotoxin-induced tonico-clonic convulsion. One hour after the

administration of distilled water and extract, 4 mg/kg of picrotoxin (RBI ® Research Biochemicals Incorporated) dissolved in distilled water were injected intra peritoneally to each animal. The animals were, then, put in individual cages for 30 minutes observation. The latency time and the duration of any convulsion sign were noted (Akula *et al.*, 2007).

## 5. Expression and analysis of results

Results were expressed as ( $m \pm \bar{\sigma}$ ), with  $n = 6$ , the means were compared using Student 't' test. The difference is considered as significant for  $P < 0.05$  when compared with control group (\*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ ).

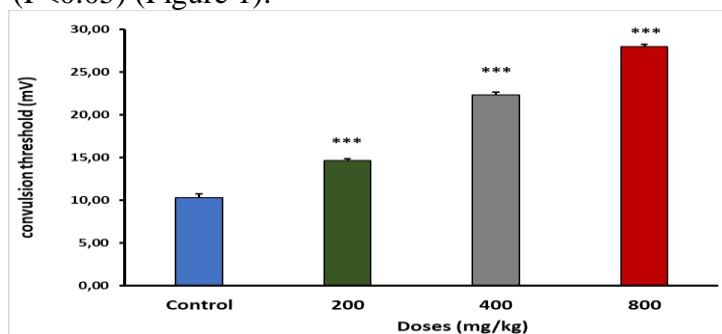
## Results

### 1. Results of the phytochemical screening

The phytochemical screening revealed the presence of high concentration of alkaloids, and small amount of reducing sugars, anthocyanins, tannins, flavonoids, steroids and terpenoids.

### 2. Effect of *A. conyzoides* activity on convulsion threshold

Oral administration of *A. conyzoides* hydroalcoholic extract increases the convulsion threshold in mice. The control group animals reacted to an electric shock of  $10.3 \pm 0.5$  mV, versus  $14.6 \pm 0.2$ ,  $22.3 \pm 0.3$  and  $28.1 \pm 0.2$  mV for the animals treated with the extract at doses 200, 400 and 800 mg/kg respectively ( $P < 0.05$ ) (Figure 1).

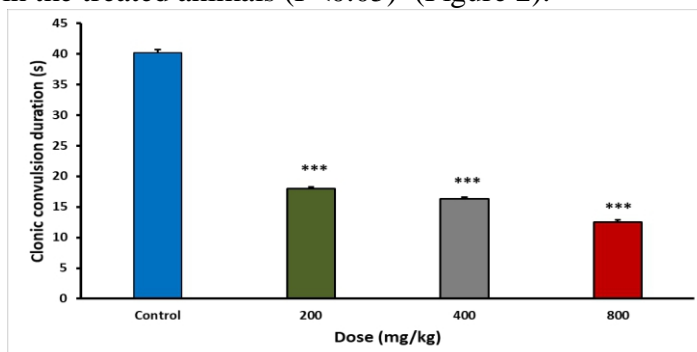


**Figure 1.** Single electric shock induced convulsion threshold in control group animals and animals given *A. conyzoides* hydroalcoholic extract at 200, 400 and 800 mg/kg administered orally ( $m \pm \bar{\sigma}$ ;  $n=6$ ; \*\*\*  $P < 0.001$  as compared to control group).

### 3. Effect of *A. conyzoides* on the duration of picrotoxin-induced clonic convulsion

Administered intra peritoneally, picrotoxin induces generalized convulsion. Hydroalcoholic extract of *A. conyzoides*, administered orally at doses 200, 400 and 800 mg/kg reduces the duration of clonic convulsion, from

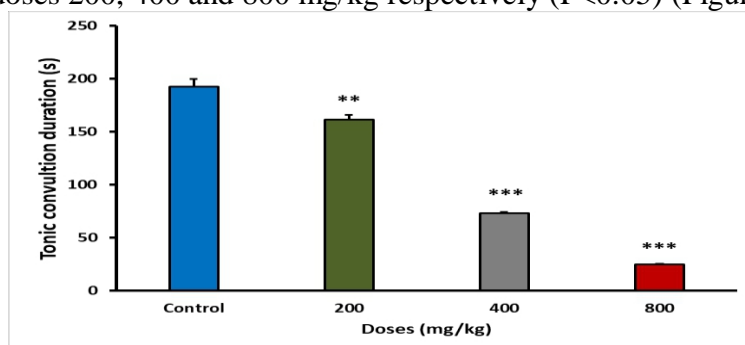
$40.1 \pm 0.5$  s in the control group to  $18.2 \pm 0.2$ ,  $16.3 \pm 0.2$  and  $12.5 \pm 0.3$  s respectively in the treated animals ( $P < 0.05$ ) (Figure 2).



**Figure 2.** Duration of picrotoxin-induced clonic convulsion in control group animals and animals treated with *A. conyzoides* hydroalcoholic extract at 200, 400 and 800 mg/kg administered orally ( $m \pm \bar{\sigma}$ ;  $n=6$ ; \*\*\*  $P < 0.001$  as compared to control group).

#### 4. Effect of *A. conyzoides* on the duration of picrotoxin-induced tonic convulsion

Hydroalcoholic extract of *A. conyzoides*, administered orally, decreases the duration of tonic convulsion induced by picrotoxin administered intra peritoneally. The animals in the control group convulse during  $192.5 \pm$  s versus  $161 \pm 4.2$ ,  $73.2 \pm 1.0$  and  $24.8 \pm 0.3$  s in the animals treated with the extract at doses 200, 400 and 800 mg/kg respectively ( $P < 0.05$ ) (Figure 3).



**Figure 3.** Duration of picrotoxin-induced tonic convulsion in control group animals and animals treated with *A. conyzoides* hydroalcoholic extract at 200, 400, and 800 mg/kg administered orally ( $m \pm \bar{\sigma}$ ;  $n=6$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$  as compared to control group).

## Discussion

*Ageratum conyzoides* hydroalcoholic extract was tested on electric shock and picrotoxin induced convulsion in mice. Administered orally, it reduces seizure crisis. It increases the seizure threshold in mice and decreases

the duration of clonic and tonic convulsion induced by picrotoxin administered intra peritoneally.

In this study, electric shock on mice ear lobe was used because it induces convulsion in small animals *in vivo* (Gerstner *et al.*, 2014). Electric shock increases sodium and potassium conductance which leads to tonic and clonic convulsions. Increasing electric shock intensity facilitates opening of voltage-dependent ionic channels (Kampa *et al.*, 2004; Lockman & Fisher, 2009). Administered before electric shock, *A. conyzoides* hydroalcoholic extract increases neuron threshold thereby protecting animals from convulsion. This may be due to the neuronal membrane stabilizing or muscle contraction inhibition. Threshold increase could be due to ionophore blockage inhibiting depolarization like carbamazepine or phenytoin which blocks sodium channels (Armijo *et al.*, 2005). Potassium channel blockage may also be involved in this mechanism. Molecules in the extract might block potassium channels which leads to threshold increase and stabilizing neuron membrane (Holmes & Zhao, 2008). It is also possible that an active compound in *A. conyzoides* blocks calcium voltage opening channels, therefore inhibits glutamate release from presynaptic neuron and muscle contraction (Krishnan & Bazhenov, 2011).

The central nervous system's functions depend on a dynamic balance between exciting and inhibiting systems, where GABA is the most important in the inhibiting system and glutamate in the exciting system. An imbalance between the two mediators, in favour of excitatory system, leads to convulsion crisis (Raimondo *et al.*, 2015). Injected intra peritoneally, picrotoxin gets to the central nervous system and blocks GABA receptors decreasing  $\text{Cl}^-$  input, thereby increasing neuron excitability, which leads to seizure crisis with tonic and clonic convulsion (Barnard *et al.*, 1998; Goutman & Calvo, 2004). Since oral administration of *A. conyzoides* extract inhibits seizure crisis, one can advance the hypotheses whereby *A. conyzoides* extract might block picrotoxin action on GABA receptors, increase pre-synaptic release of GABA or inhibit degradation of GABA in synaptic cleft like vigabatrin by inhibiting GABA-aminotransferase. In this case, the increase of GABA concentration in the synaptic cleft leads to increase in  $\text{Cl}^-$  input, stabilizing the membrane, thereby inhibiting seizure crisis (Macdonald & Kelly, 1995; Czapiński *et al.*, 2005).

Furthermore, AMPA receptor activation is responsible for rapid excitation responses, and glutamate fixation on this receptor increases  $\text{Na}^+$  neuronal input, leading to neurone hyperexcitability. This provokes seizure crisis (Twomey *et al.*, 2017). An active compound in *A. conyzoides* extract might bind to this receptor and inhibits seizure crisis.

Another possibility is that an active compound in *A. conyzoides* extract might inhibit glutamate release, by binding to NMDA receptor. Decreases of  $\text{Ca}^{2+}$  input in post synaptic neuron will decrease recycling of glutamate in

presynaptic neuron. This will lead to decrease of glutamate released into the synaptic cleft (Wang, 2013; Guo *et al.*, 2017).

Flavonoids, alkaloids or terpenes might be responsible for the anticonvulsant activity of *A. conyzoides* hydroalcoholic extract. As reported by Rasilingam *et al.* (2009), gossypin, a flavonoid isolated from *Hibiscus vitifolius* L. (Malvaceae) protects mice from MES-induced seizure; similarly flavonoids isolated from *Nauclea latifolia* Sm. (Rubiaceae) protect mice against MES-induced seizures through GABA or by inhibiting sodium channels (Bum *et al.*, 2009), and the flavonoid, galangin, isolated from *Acorus calamus* L. (Acoraceae) act through GABA receptor (Jayaraman *et al.*, 2010). Alkaloids also have been reported to be responsible for anticonvulsant activity. For example: Da Cruz *et al.* (2013) reported that the alkaloid piperine protects mice from MES-induced seizure through GABA receptor; while Riberio and Laite (2003) have noted that the alkaloid nantenine decreases  $Ca^{2+}$  influx in post synaptic neuron. Chen *et al.*, (1996) also reported that the alkaloid ibogaine protects mice from MES-induced seizure by blocking NMDA receptor. The anticonvulsant activity of terpenes also have been reported. According to De Sousa *et al.* (2006), the terpene citronellol has a protective activity against MES-induced seizure by  $Na^{+}$  channel inhibition; while linalool, a terpene isolated from *Lavandula officinalis* protects mice from MES-induced seizure crisis by inhibiting NMAD receptor (ARZI *et al.*, 2011).

## Conclusion

The results of this work show that *A. conyzoides* hydroalcoholic extract possesses anticonvulsant effect. The flavonoids, alkaloids or terpenes might be responsible for its anticonvulsant activity. At this stage, it is difficult to determine exactly what the active compounds are. Further studies are necessary to identify them and determine their mechanisms of action.

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